Supporting Information

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SI Text

Phylogenetic Analysis. A BLASTP search was performed with NCBI BLAST version 2.2.19 (1) using the amino acid sequence of PpCLF as a query. The target dataset was "nr" of November 30, 2008. Default parameters were used. The hit sequences were collected, but non "refseq" sequences from *A. thaliana* and *Oryza sativa* were removed. The retrieved amino acid sequences were aligned with "einsi" of MAFFT version 6.611b (2). The alignment was converted to NEXUS format and edited with MacClade version 4.08 (3) to exclude sites containing gaps or ambiguity. Some sequences were very closely related to other sequences in preliminary phylogenetic analyses and one of the similar sequences was selected in the matrix. The remaining sequences were written to the PHYLIP format. The alignment was investigated for the maximum likelihood tree using a parallelized multithread version of RAxML version 7.0.4 (4, 5). The best fit model was selected as JTT (6) using a perl script (http://icwww.epfl.ch/~stamatak/index-Dateien/software/ProteinModelSelection.pl). This script computed the likelihood of a MP tree under the BLOSUM62 (7), CPREV (8), DAYHOFF (9), DCMUT (10), JTT (6), MTMAM (11), MTREV (12), RTREV (13), VT (14), and WAG (15) models and their variants using empirical amino acid frequencies. The ML trees were searched with the rapid bootstrap MP and ML searches (5) with "-f a -x 323 -# 1000" options. A full bootstrap for 1,000 replicates was performed with "-f i -b 327 -# 1000" option, and the occurrence of each branch on the best tree was calculated with "-f b" option.

Construction of Plasmids for Deletion and Citrine Knock-In. Primer sequences are listed in Table S1. To delete the *PpCLF* gene, the 3′ genomic DNA fragment of *PpCLF* was amplified using the PpCLF-3′g-F1 and PpCLF-3′g-R1 primers. The amplified 3′ fragment was inserted into pTN182 (16, 17) (AB267706) digested with *Sma*I, and named pCLF-*npt* II-dis-3′. The 5′ genomic DNA fragment of *PpCLF* was amplified using the PpCLF-5′g-F2 and PpCLF-5′g-R1 primers. The amplified 5′ fragment was inserted into pCLF-*npt* II-dis-3′, digested with *Eco*RV, and named pCLF-*npt* II-dis. A PCR fragment amplified using the pCLF-*npt* II-dis plasmid as template with the PpCLF-5′g-F2 and PpCLF-3′g-R1 primers was used for gene targeting.

To delete the *PpCLF* gene in the background of the MKN4-GUS-3 line (17) or the PpLFY2-GUS-1 line (18), the 3′ genomic DNA fragment of *PpCLF* was amplified using the PpCLF-3′g-F1 and PpCLF-3′g-R1 primers. The amplified fragment was inserted into pTN86 (AB267705) (16, 17) digested with *Sma*I, and named pCLF-aphIV-dis-3′. The 5′ genomic DNA fragment of *PpCLF* was amplified using the PpCLF-5′g-F2 and PpCLF-5′g-R1 primers. The 5′ amplified fragment was inserted into the pCLF-aphIV-dis-3′ digested with *Eco*RV, and named pCLF-aphIV-dis. A PCR fragment amplified using the pCLF-aphIV-dis plasmid as template with the PpCLF-5′g-F2 and PpCLF-3′g-R1 primers was used for gene targeting.

To construct PpCLF-Citrine lines, the 3′ genomic region including the 3′ untranslated region of *PpCLF* just after the stop codon was amplified using the pCLF-*npt* II-dis plasmid as template with the PpCLF-3′g-F1 and PpCLF-3′g-R1 primers and inserted into *Sma*I-digested pCit-npt, which contains the coding region for Citrine (19) with the start codon replaced by the sequence TTG, a nopaline synthase terminator, and a *npt* II cassette. This plasmid was named pCLF-Cit-3′. The genomic DNA fragment of the *PpCLF* coding region just before the stop codon was amplified using the PpCLF-CTR-F2 and PpCLF-CTR-R1 primers. The amplified fragment was inserted into pCLF-Cit-3′, digested with *Eco*RV, and named pCLF-Cit. A DNA fragment obtained from the digestion of pCLF-Cit with *Xho*I and *Xba*I was used for gene targeting.

To delete the *PpFIE* gene, the 3′ genomic DNA fragment of *PpFIE* was amplified using the PpFIE-3′g-F1 and PpFIE-3′g-R1 primers. The amplified fragment was inserted into pTN182 (16, 17) (AB267706) digested with *Sma*I, and named pFIE-*npt* II-dis-3′. The 5′ genomic DNA fragment of *PpFIE* was amplified using the PpFIE-5′g-F1 and PpFIE-5′g-R1 primers. The 5′ amplified fragment was inserted into the pFIE-*npt* II-dis-3′ digested with *Eco*RI, and named pFIE-*npt* II-dis. A fragment containing an *npt* II cassette of pFIE-*npt* II-dis was replaced with an aphIV cassette from pTN86 (16, 17) (AB267705) with *Eco*RV to make pFIE-aphIV-dis. A PCR fragment amplified using the pFIE-aphIV-dis plasmid as template with the PpFIE-5′g-F1 and PpFIE-3′g-R1 primers was used for gene targeting.

Construction of Plasmids for Heat-Shock Induction. To construct HSP-PpCLF-Cerulean/ppclf-del lines, a PCR fragment amplified using the pCLF-*npt* II-dis plasmid as template with the PpCLF-5′g-R1 and PpCLF-3′g-F1 primers was self-ligated and named pCLF-dis; most of the *PpCLF* coding region is deleted in this plasmid. A cDNA fragment of a full-length *PpCLF* coding sequence with the start codon and without the stop codon was amplified using the pPpCLFcDNA plasmid as template with the PpCLF-cacc-ATG and PpCLF-without-stop primers. The amplified fragment was cloned into the pENTR/D-TOPO cloning vector (Invitrogen) and named pCLF-CDS. The plasmid pHSP-CLF was constructed using the LR Clonase (Invitrogen) reaction between pCLF-CDS as the entry vector and pPIG1HGC as the destination vector. A PCR fragment amplified using the pCLF-dis plasmid as template with the PpCLF-5′g-F2 and PpCLF-3′g-R1 primers and pHSP-CLF digested with *Pme*I were co-transformed into the wild type for gene targeting.

DNA Gel-Blot Analysis. DNA gel-blot analysis was performed as described previously (20). PCR fragments amplified using genomic DNA of the wild type as templates with the PpCLF-3′g-F1 and PpCLF-3′g-R1 primers, PpCLF-CTR-F2 and PpCLF-CTR-R1 primers, and PIG1bR-213 and PIG1bR-956 primers were used as the PpCLF-3′ probe, the PpCLF-coding probe, and the PIG1–5′ probe, respectively.

Flow Cytometry To Measure DNA Content. Protonemata or sporophyte-like tissues were chopped with a razor blade in extraction buffer containing 10 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 0.1% Triton X-100, and 100 mg/mL RNase A and incubated for 20 min on ice. All preparations were filtered through a 20 μm Celltrix filter (PARTEC) and stained with propidium iodide (PI) solution containing 1 mg/mL PI (Sigma) in 1.25% DABCO solution (Sigma). Flow cytometry was performed on an EPICS XL cytometer (Beckman Coulter).

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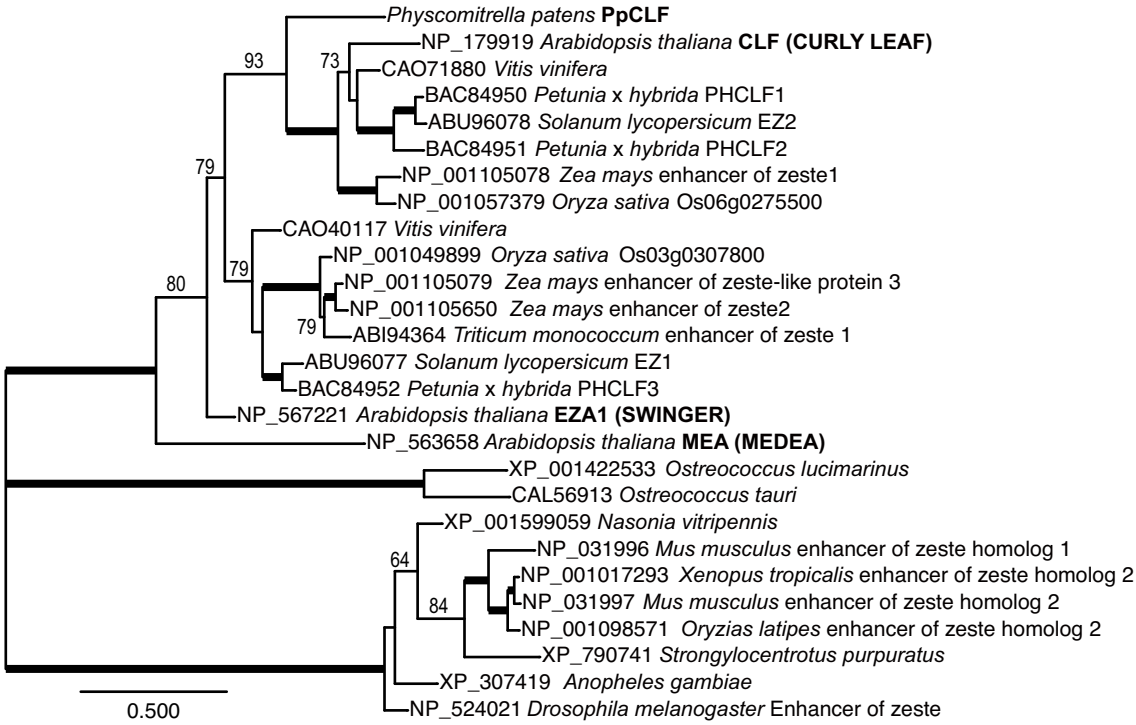

Fig. S1. A maximum-likelihood tree of representative *Enhancer of Zeste*-like genes. Bootstrap probabilities of more than 60% but less than 95% are given above the branches; when the probability is at least 95%, the branch is drawn with a thick line. Horizontal branch length is proportional to the estimated evolutionary distance. Accession number and species are followed by the gene product name, if available.

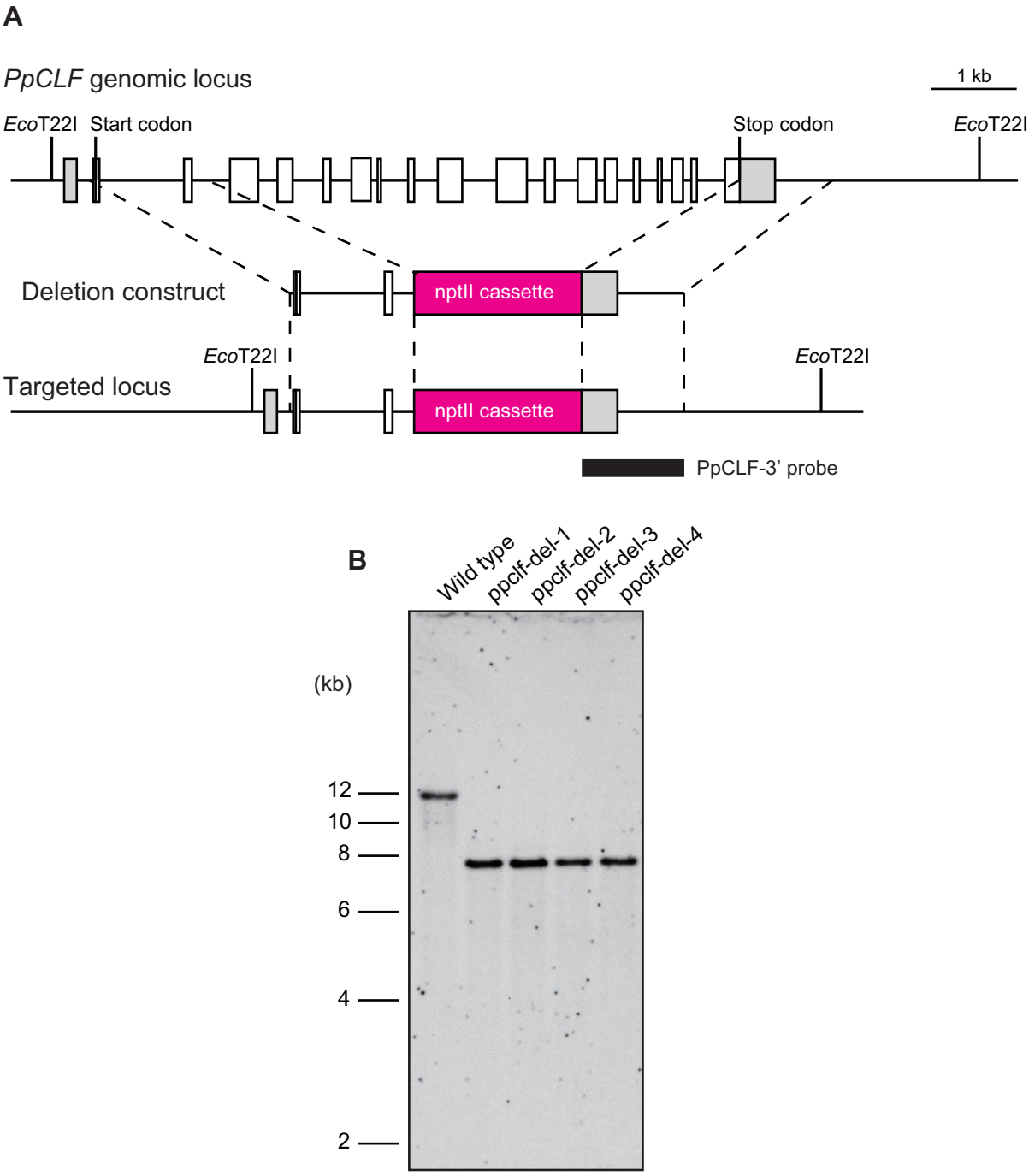

Fig. S2. Generation of the PpCLF-deletion mutant line in wild-type plants. (*A*) Schematic of the deletion of the *PpCLF* genomic locus. White boxes represent *PpCLF* coding sequences. Gray boxes indicate the 5′ and 3′ untranslated regions. The PpCLF-3′ probe used for DNA gel-blot analysis is indicated. The pink box indicates the neomycin phosphotransferase II expression cassette (*npt* II) (16). (*B*) DNA gel-blot analysis of deletion lines. Genomic DNA of the wild type and the deletion lines was digested with *Eco*T22I and hybridized with the PpCLF-3′ probe.

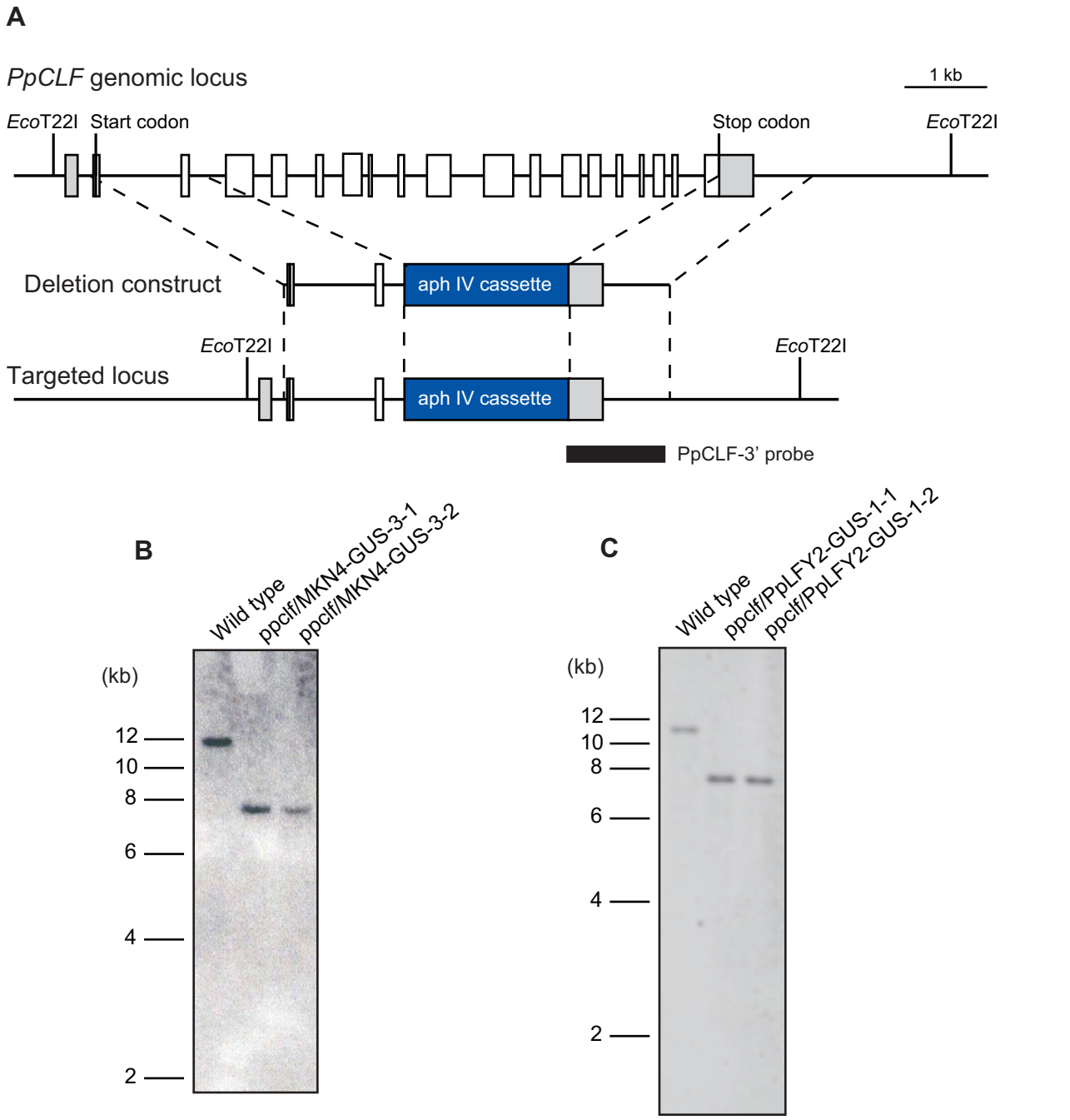

Fig. S3. Generation of the PpCLF-deletion lines in the MKN4-GUS-3 (17) and PpLFY2-GUS-1 (18) background. (*A*) Schematic of the deletion of the*PpCLF* genomic locus. Schematic is drawn as explained for Fig. S2. The blue box indicates the aminoglycoside phosphotransferase IV (aph IV) expression cassette (pTN86 [AB267705]). (*B* and *C*) DNA gel-blot analyses of deletion lines in the MKN4-GUS-3 (*B*) and PpLFY2-GUS-1 (*C*) background. Genomic DNA of the wild type and deletion lines was digested with EcoT22I and hybridized with the PpCLF-3′ probe.

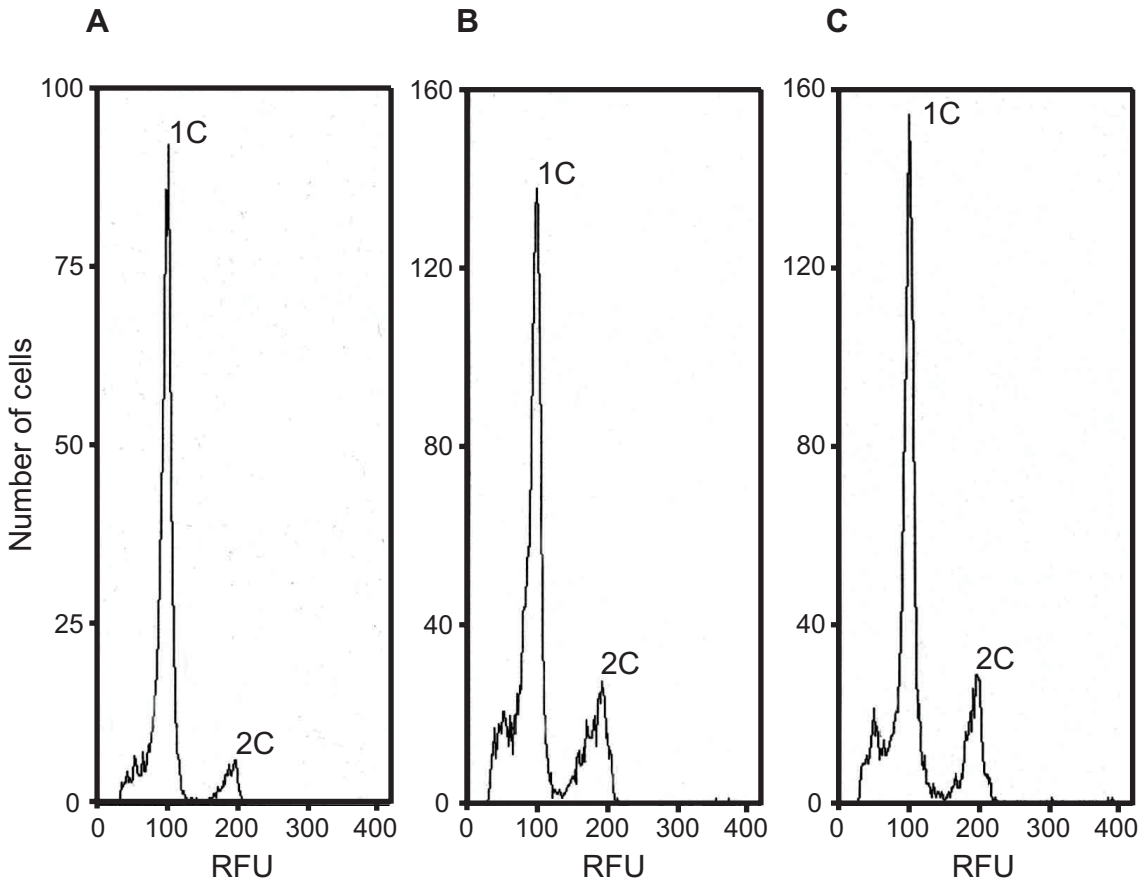

Fig. S4. DNA content of the PpCLF-deletion mutant lines. The ploidy of wild type (*A*), ppclf-del-2 (*B*), and ppclf-del-3 (*C*) grown in white light for 2 weeks was analyzed by flow cytometry. RFU, relative fluorescence units.

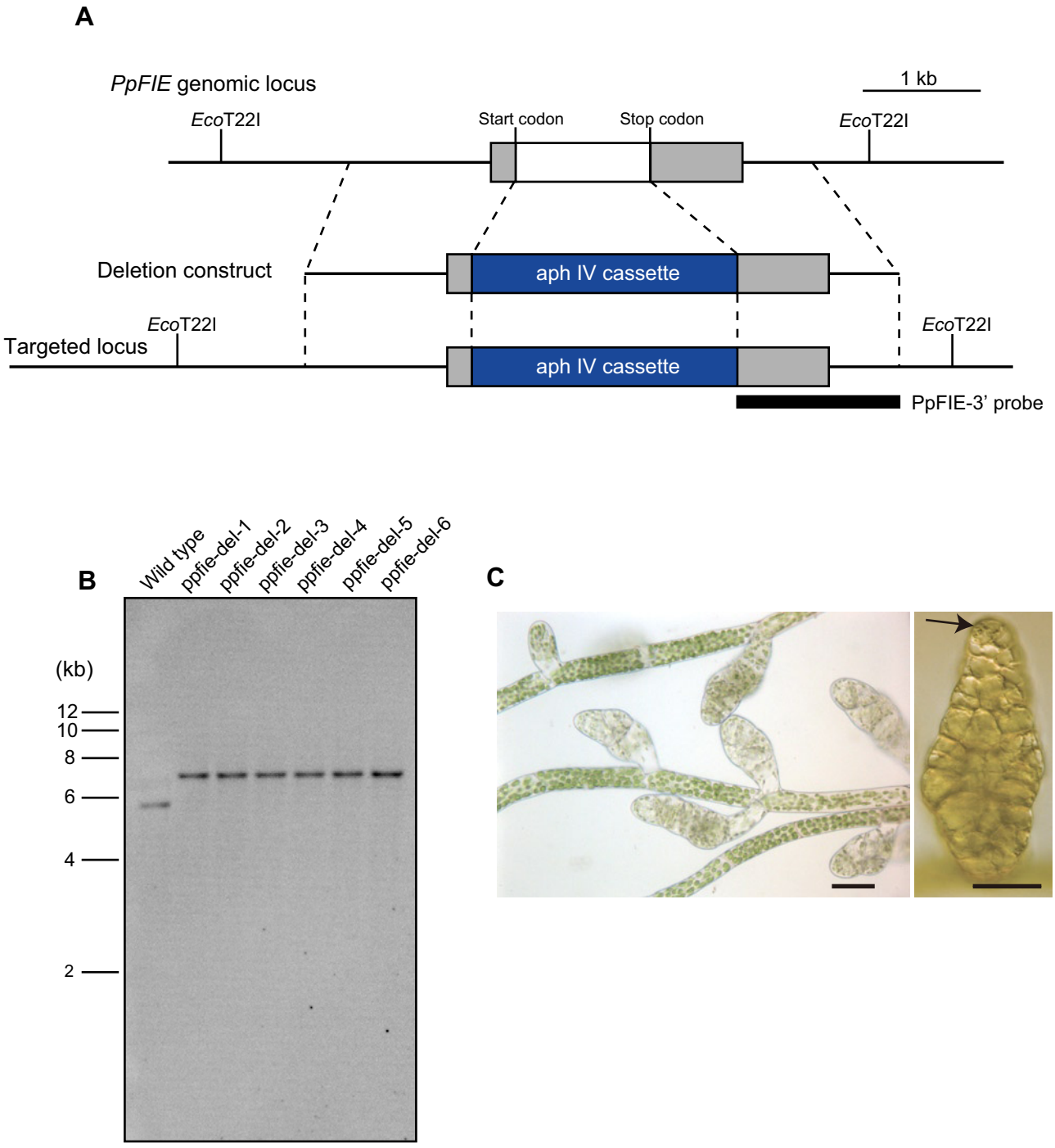

Fig. S5. Generation of the PpFIE-deletion lines in wild type plants and phenotype of the lines. (*A*) Schematic of the deletion of the *PpFIE* genomic locus. White boxes represent *PpFIE* coding sequences. Gray boxes indicate the 5′ and 3′ untranslated regions. The PpFIE-3′ probe used for DNA gel-blot analysis is indicated. The blue box indicates the aminoglycoside phosphotransferase IV (aph IV) expression cassette (pTN86 [AB267705]). (*B*) DNA gel-blot analysis of deletion lines. Genomic DNA of the wild type and the deletion lines was digested with *Eco*T22I and hybridized with the PpFIE-3′ probe. (*C*) Protonemata of ppfie-del-2 grown in white light for 2 days after 8 days of culture in red light (left panel) and a sporophyte-like body formed on the ppfie-del-2 protonema grown under white light for 7 days (right panel). An arrow indicates an apical cell. (Scale bars, 50 μm.)

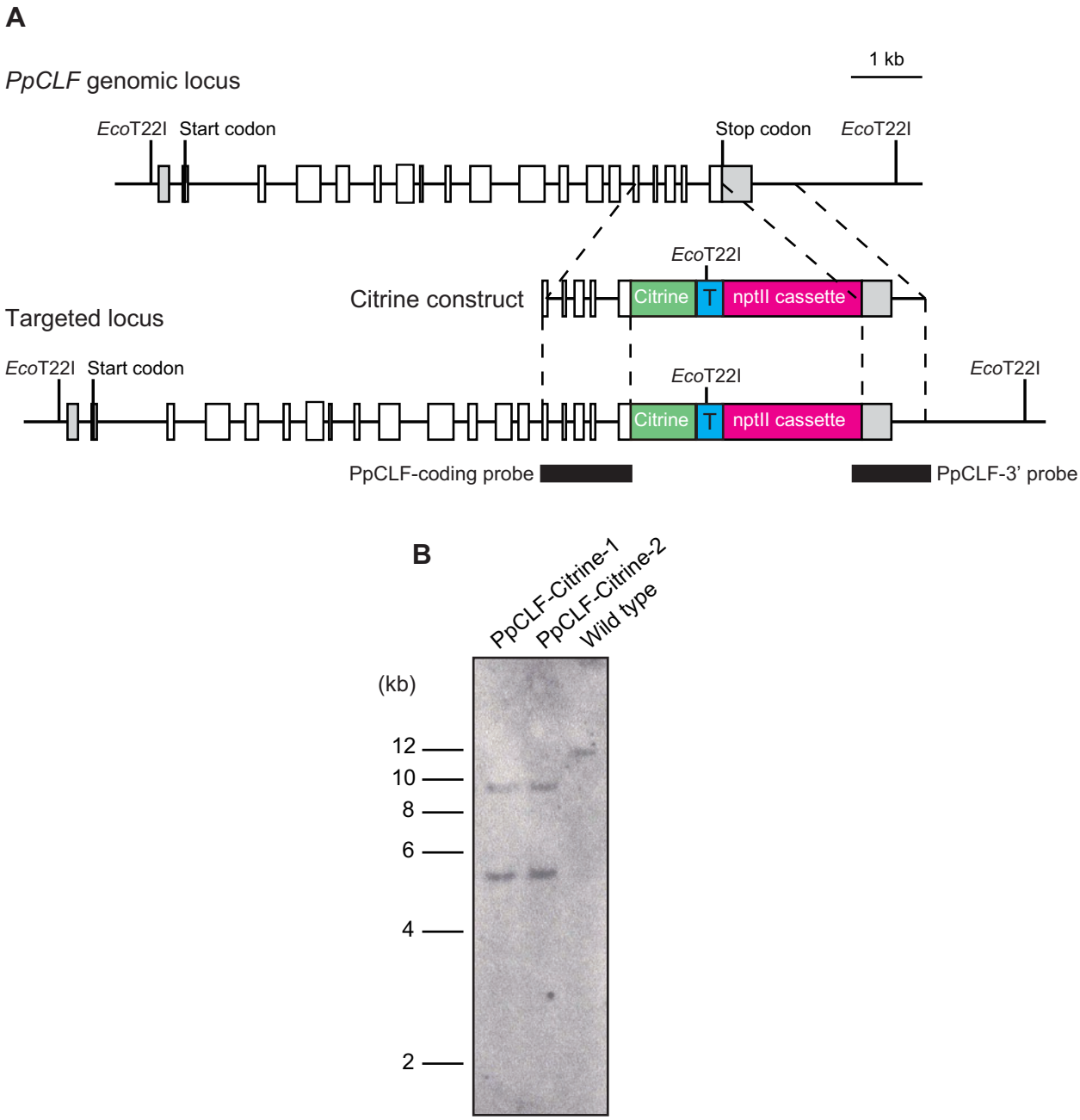

Fig. S6. Generation of the PpCLF-Citrine transformant. (*A*) Schematic of the targeting of the *PpCLF* genomic locus. Schematic is drawn as explained for Fig. S2. Green, pink, and blue boxes indicate the *Citrine* gene (Citrine) (19), the neomycin phosphotransferase II expression cassette (*npt* II) (16), and the nos terminator (T) (16). (*B*) DNA gel-blot analysis of the PpCLF-Citrine transformants. Genomic DNA of the wild type and the transformants was digested with *Eco*T22I and hybridized with both PpCLF-coding and PpCLF-3′ probes.

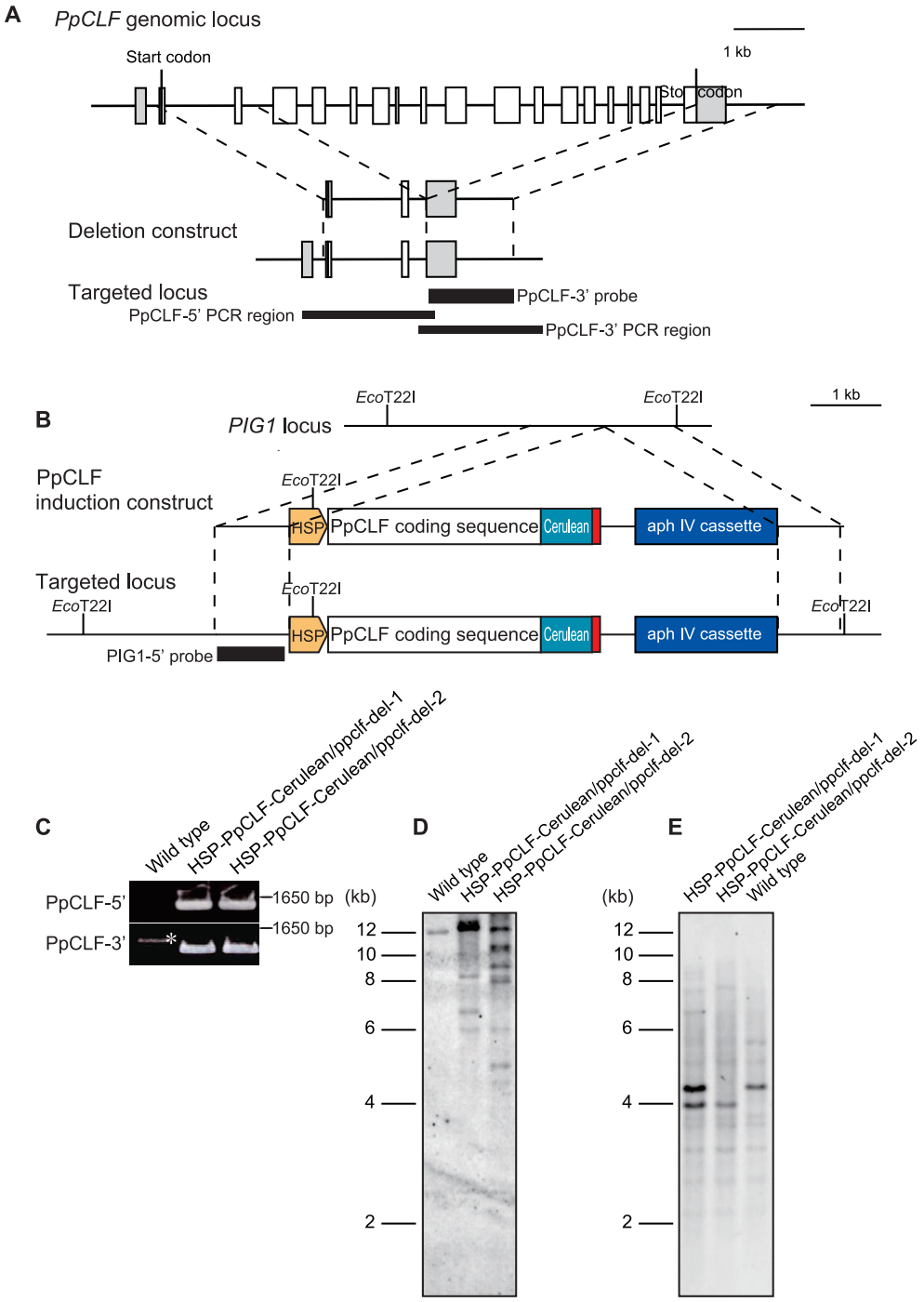

Fig. S7. Generation of the HSP-PpCLF-Cerulean transformant in the background of the PpCLF-deletion mutant line. (*A*) Schematic of the disruption of the *PpCLF* genomic locus. Schematic is drawn as explained for Fig. S2. The PpCLF-3′ probe used for DNA gel-blot analysis is indicated. The PpCLF-5′ PCR region and PpCLF-3′ PCR region used for genomic PCR analysis are indicated. (*B*) Schematic of insertion of the PpCLF induction construct into the *PIG1* targeting locus. Yellow, white, light blue, red, and blue boxes indicate the soybean heat-shock Gmhsp17.3B promoter (HSP) (21), the full-length *PpCLF* coding sequence, the Cerulean gene (22), the pea rbcS terminator, and the aminoglycoside phosphotransferase IV (aph IV) expression cassette (pTN86 [AB267705]). The PIG1–5′ probe used for DNA gel-blot analysis is indicated. (*C*) Genotyping by PCR analysis of the *PpCLF* genomic locus. The PpCLF-5′ PCR region and PpCLF-3′ PCR region of the wild type and the transformants were amplified with the PpCLF-5′g-F2 and PpCLF-3′g-R3 primers and PpCLF-5′g-F3 and PpCLF-3′g-R2 primers, respectively (Table S3). Asterisk indicates a non-specific band. (*D*) DNA gel-blot analysis of the *PpCLF* genomic locus. Genomic DNA of the wild type and the deletion mutant lines was digested with *Eco*T22I and hybridized with the PpCLF-3′ probe. (*E*) DNA gel-blot analysis of the *PIG1* genomic locus. Genomic DNA of the wild type and the deletion mutant lines was digested with *Eco*T22I and hybridized with PIG1–5′ probe.

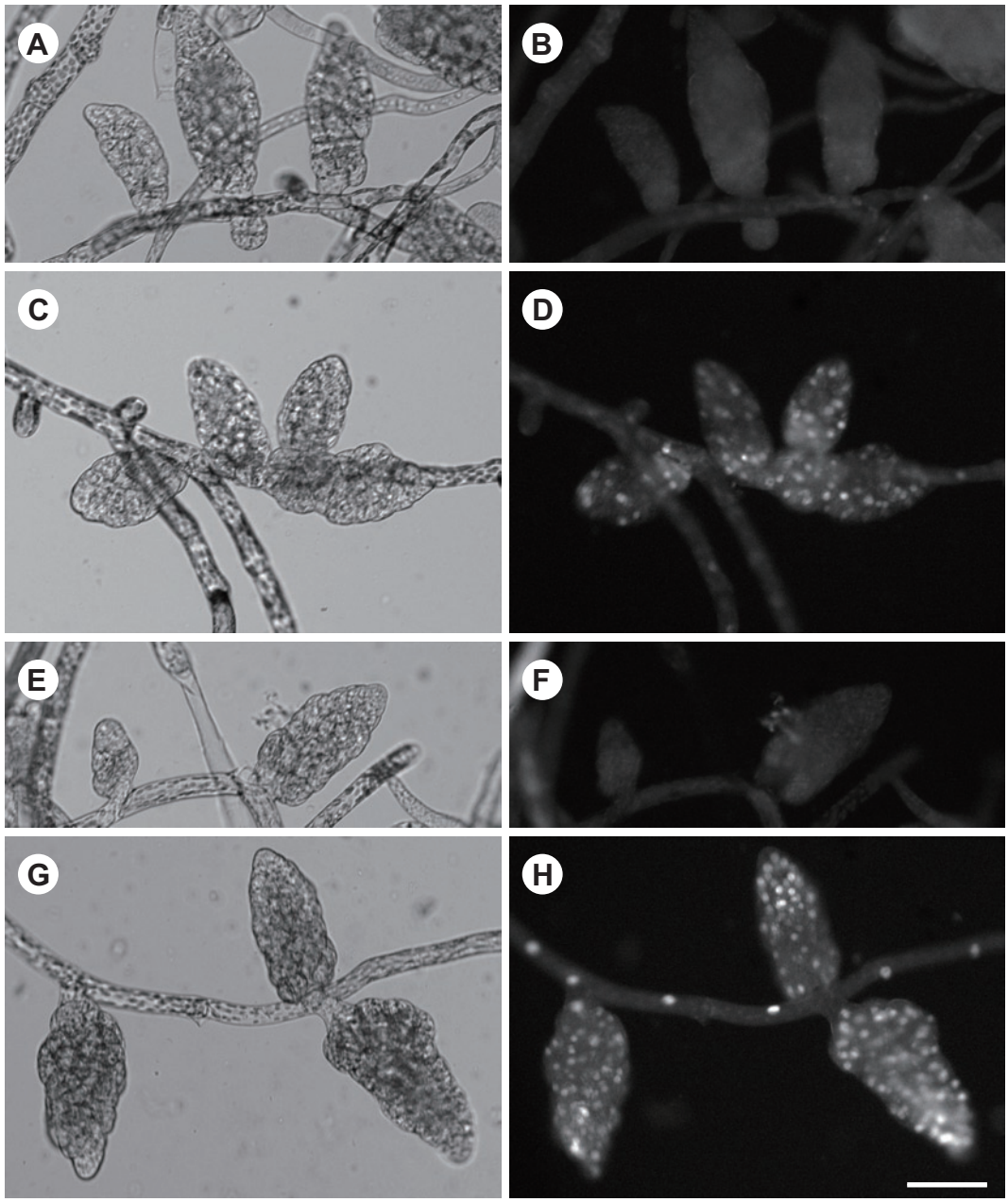

Fig. S8. Fluorescence of the induced PpCLF-Cerulean fusion protein upon heat shock in the HSP-PpCLF-Cerulean/ppclf-del deletion lines. The HSP-PpCLF-Cerulean/ppclf-del-1 (*A*–*D*) and -2 (*E*–*H*) lines were cultivated with heat shock at 37 °C for 1 h every 12 h (*C*, *D*, *G*, and *H*), or without heat shock (*A*, *B*, *E*, and *F*). Bright-field images (*A*, *C*, *E*, and *G*) and fluorescence images with CFP filter (*B*, *D*, *F*, and *H*) are shown. (Scale bar, 100 μm.)

Table S1. Average number of protonemata, gametophores, or sporophyte-like bodies formed as side branches per protonema with or without exogenous N^6-benzylaminopurine (BAP)

Genotype	0.5 μM BAP	Protonemata	Gametophores	Sporophyte-like bodies	Not specified*
Wild type	minus	4.9 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Wild type	plus	2.3 ± 0.3	6.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
ppclf-del-2	minus	0.7 ± 0.1	0.0 ± 0.0	5.8 ± 0.3	0.1 ± 0.1
ppclf-del-2	plus	0.6 ± 0.2	0.0 ± 0.0	4.5 ± 0.3	0.2 ± 0.1
ppclf-del-3	minus	0.6 ± 0.1	0.0 ± 0.0	5.6 ± 0.3	0.1 ± 0.1
ppclf-del-3	plus	0.5 ± 0.1	0.0 ± 0.0	4.3 ± 0.3	0.1 ± 0.1

Side branch initial cells formed at the 6th to 12th protonemal cells from an apical cell were examined. Data report mean ± SD for 30 protonemata.
*Not specified tabulates primordia that could not be identified

Table S2. Average number of protonemata, gametophores, or sporophyte-like bodies formed as side branches, per protonema, with heat-shock treatment

Genotype	Protonemata	Gametophores	Sporophyte -like bodies	Not specified*
Wild type	3.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HSP-PpCLF-Cerulean/ppclf-del-1	1.8 ± 0.3	1.9 ± 0.3	0.1 ± 0.1	0.3 ± 0.1
HSP-PpCLF-Cerulean/ppclf-del-2	1.4 ± 0.3	2.4 ± 0.4	0.0 ± 0.0	0.1 ± 0.1
ppclf-del-3	0.7 ± 0.2	0.0 ± 0.0	4.6 ± 0.3	0.1 ± 0.1

Side branch initial cells formed at the 6th to 12th protonemal cells from an apical cell were examined. Data report mean ± SD for 30 protonemata.
*Not specified tabulates primordia that could not be identified.

Table S3. PCR primers

Primer	Sequence (5′–3′)
PpCLF-5′g-F2	GCGACGAGAGGAACAAGGTTTCGC
PpCLF-5′g-R1	TGCTACCAATATCGAGAACCCCAGCAG
PpCLF-3′g-F1	GTGGCTCATTTACTGTTGCAGTTCAGGTC
PpCLF-3′g-R1	GCACATAGCGCAGTTGCAAACGCTC
PpCLF-CTR-F2	ACATGTAAAGCCGGCAGTGGAGTA
PpCLF-CTR-R1	AGCAACTTTCTGTGCTCGTCCACC
PpFIE-5′g-F1	GTTGGGATGCACTGGTAATAGTTGGAACC
PpFIE-5′g-R1	AGCTGTGGCTGAACCCTAAACCTCCA
PpFIE-3′g-F1	GATGATCGTGGATATCTGGAGCCAATTTC
PpFIE-3′g-R1	TGGTCCAATTAGGAAACTAATGGTTGATTCC
PpCLF-cacc-ATG	CACCATGGCGTCCTCCAGCTACGCCATC
PpCLF-w/o-stop	AGCAACTTTCTGTGCTCGTCCACCGG
PpCLF-5′g-F1	GGAGGAGCTTCTTTGCTGTTTGCTGG
PpCLF-5′g-F3	CTGCTGGGGTTCTCGATATTGGTAGCA
PpCLF-3′g-R2	ACGATCCCAGGCAGGAAGCATTGC
PpCLF-3′g-R3	GACCTGAACTGCAACAGTAAATGAGCCAC
KSP-PIG1bLf1	GGGGTACCTGCAGGTTTAAACACATTTTTTATGTGGGCCGTTGTAGA
Xh-PIG1Lr1	CCGCTCGAGTGTATTCTATTTGATTGATAAGAAAAATG
Xb-PIG1Rf1	GCTCTAGAAAACATGAATAACCAAATTAAAATATTAATAATTC
SSP-PIG1bRr1	ACGAGCTCCTGCAGGTTTAAACTAAGATTTCTATGCACGGATAGCAAC
PIG1bR-213	TTTTCCAAACAGAAAACTTCCA
PIG1bR-956	AAAACATGATATCTTTTGCAAGG